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Development of the adrenal glands in the embryo chick

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THE DEVELOPMENT of the ADRENAL GLANDS in the EMBRYO CHICK

by

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THE DEVELOPMENT OF THE ADRENAL GLANDS IN THE EMBRYO CHICK

INTRODUCTION

The development of the adrenal glands has been the object of a number of investigations covering a long period of years. Investigations have been carried on by investigators who were and are authorities in their field of endeavor, yet there seems to be no very general agreement in the conclusions at which they have arrived. This is more true of the investigations concerning aves than any other group of animals. This is no doubt the result of the fact that there is no well defined division between the medullary substance and the cortical substance of the adrenal glands of aves. Due to the lack of a well defined division of these substances earlier investigators were divided in their opinions concerning the origin of both medullary and cortical substances. A list of some of the views held concerning their origin will serve to give the reader an idea of the perplexity confronting the student from his review of literature. Some of these views are as follows:

1. The theory was held that the adrenal glands were derived from one substance, namely the mesenchyme. It was thought that the same material which formed the cortical substance later differentiated to form the medullary substance.

2. (a) The theory was held that the medullary and cortical substances were of separate origin, and that the medullary

cords were derived from the sympathetic ganglia while the cortical cords were derived from the sexual cords.
(b) That the medullary cords were derived from ingrowths of the sympathetic system while the cortical cords were proliferations from an ingrowth and budding off of the peritoneal epithelium.

From the preceding statements conclusions are readily drawn that some of the earlier investigators derived the entire gland from one substance while others derived it from two substances. The former is called the homogenous theory and the latter the heterogenous theory. These investigators who upheld the homogenous theory were Valenti, Rathke, Goodsir, Gray, Von Brunn, Sedgwick, and Gottschau. These men thought the adrenals to be of mesodermic origin and that certain cells of the same substance became differentiated to form the medulla. Janosik, Valenti, and Mihalcovics thought them to be originated from the germinal epithelium. Bischoff, His, Waldeyer, and Aichel thought them to be originated from the epithelium of the mesonephric tubules; and finally Semon derived them from the pronephros. Those investigators who upheld the heterogenous theory were divided among themselves as to the origin of the substances. They agreed only that the gland was derived from two substances. Remak, Kolliker, Braun, Balfour, Mitsukuri, and Minot thought the cortical substance to be derived from the mesoderm. Inaba, Fusari,

Srdinko, Wiesel, and Brauer thought the cortical cords to be of epithelial origin but were unsettled as to whether they were derived from the germinal or peritoneal epithelium. Weldon and Hoffman thought the cortical substance to be derived from the epithelium of the tubules of the mesonephros while Rabl and Minervini thought it to be derived from the pronephros. The reader is at once aware of the fact that a diversity of opinions are held concerning the origin of the cortical substance of the adrenal glands.

The medulla was thought to be formed by differentiated cells of the mesenchyme by Gottschau and Minot. Von Mihalcovics, working with reptiles, came to the conclusion that the medulla was derived from the germinal epithelium and this theory was substantiated by Janosik and Valenti both working with the adults and embryos of birds. Leydig, working with fish, came to the conclusion that the medulla was of sympathetic origin. Balfour in his work on elasmobranch fishes shows conclusively that the medulla is of sympathetic origin and since his time investigators have found this to be true. Poll, from his extensive review of literature and work on mammals, birds, and reptiles came to the conclusion that the cortical substance is derived from the peritoneal epithelium and that the medulla is formed from the periphery of the sympathetic nervous system. Soulie working with birds and Kuntz working with turtles also came to these conclusions.

Vincent says, "That the cortex is derived from the mesoderm and the medulla from the same blastema as the sympathetic ganglia is now almost universally conceded." Lillie in his review of literature in regard to views held concerning the development of the adrenal glands says that in his estimation the veiw supported by Poll and Soulie is the logical explanation. Hence one can see that at the present time there is no definite agreement as to the origin of these substances, although there is a definite agreement that the gland is composed of two substances of different origin.

The preceding review of literature covers but a small part of the entire field of investigation. The purpose of this review is to call attention to the confusion which has existed in regard to the development of the adrenals. A complete bibliography will be found in the work of Poll for any one desiring a fuller and more comprehensive review of the subject.

The purpose of this investigation is to determine the source of the cells, both medullary and cortical, which go to form the adrenal gland; to observe the migration of cells and note any changes in size or shape during the period of formation of the gland.

The writer is pleased to acknowledge his indebtedness to Dr. R. T. Young for suggesting the topic of this investigation and for the many helpful suggestions and criticism

during the course of the experiment. The writer also wishes to acknowledge his indebtedness to Dr. M. J. Elrod for criticism and suggestions helpful to the completion of the experiment.

MATERIAL AND METHODS

The materials used for this experiment were the adult and embryos of the common hen, Gallus domesticus. The eggs were incubated artificially and taken out at regular periods every twenty-four hours. The fixatives used were: Zenker's fluid, Bouin's fluid, Regaud's fixative, plain potassium bichromate of three percent strength, Champy's fixative, and acetic alcohol. The best results were obtained from Champy's and the plain potassium bichromate in the study of cell inclusions and granules. Acetic alcohol followed by staining in Sudan III also showed the cytoplasmic and nuclear structures well.

Staining was mainly with iron haemotoxyln. The sections previously fixed to the slides by the albumen method were run down through xylol and the different grades of alcohol to water and then mordanted in iron alum for eighteen to twenty-four hours. They were then washed in distilled water and stained in iron haemotoxyln for a similar length of time. Destaining was done in iron alum. This process is rapid and needs to be watched closely under the microscope as there is danger of destaining too much. Destaining should be stopped when the structures appear pearl gray. Cellular structures show up plainly under this process and in many cases there is a beautiful display of mitochondria. The nucleus stains deeply

while the rest of the structure takes a less deep stain thus making differentiation comparatively easy. The medullary substance takes a deeper stain than the cortical cells so that both elements are readily distinguishable. This is more true of the earlier stages than those of a later period.

OBSERVATIONS

It has been previously reported that the anlagen of the cortical substance is first seen at the 96th hour. From my observations I find at the end of the third day of incubation a bud of cells being proliferated off from the peritoneal epithelium lateral to the base of the rool of the ventral mesentery. (Fig. 1.) This bud of cells is rectangular in shape. In a few cases isolated cells and secondary buds of cells can be seen migrating from the primary bud. These all migrate dorsally between the dorsal aorta and the mesonephros. In the earlier stages of development the cells rarely pass the ventral level of the dorsal aorta. They divide rapidly and pile up, forming a comparatively compact mass mesial and ventral to the mesonephros. The cells which have been budded off from the peritoneal epithelium have increased in size until they are approximately three times the size of their parent cells. They are larger and more clearly defined than those of the surrounding mesenchyme in which they are imbedded. The cytoplasm of the cortical anlagen is more granular than that of the surrounding mesenchyme cells. The nuclei are large in comparison with the nuclei of the mesenchyme cells, being approximately one-third the area of the cytoplasm. The size of the cells and volume of the nuclei are two characteristics which make the anlagen of the cortical cells recog-

nizable at this time.

Due to the compactness of the embryonic cortical tissue those cells in the central part of the bud have lost their rotundity and are rectangular or triangular in shape. This is explained by the fact that an active mitotic division is taking place continually in all the cells of the bud; hence those at the center are being subjected to pressure, while those at the periphery are still migrating and as yet do not show evidences of pressure. Those cells at the superior end of the bud are literally stretching out or elongating as they migrate and have assumed shapes which for cellular comparison may be said to be ameboid. At this stage the most noticeable appearance in regard to growth is the rapid mitotic division and subsequent change of form of the central cells of the bud.

Up to the fourth day of incubation, the cortical cells have maintained a rapid rate of division and migration, but instead of being scattered promiscuously there is not a tendency toward the formation of cords of cells. These, however, are not well established until the end of the fifth day. The cytoplasm of the cells at this time is dense and granular. The cell is somewhat smaller than that of the preceding stage, and the nucleus is smaller in proportion to the reduced amount of cytoplasm. The gland at this time occupies a well defined area lateral to the dorsal aorta and mesial to the

mesonephros.

At the end of the fifth day of incubation the gland occupies a well formed area in the same location as described above, with the exception that it has pushed slightly farther dorsally. The cords of cells are becoming well established. (Fig. 2.) Outpushings of the subcardinal vein can be seen at this time in the gland although they are very minute. The cortical cells at this stage present a striking contrast with the epithelial cells of the mesonephric tubules. The cells of the mesonephric tubules have a distinct polarity, the nuclei being situated in the distal portion of the cytoplasm with regard to the lumen, while the cortical cells do not exhibit polarity. There are two or three nuclei (generally) in each of the cortical cells, which still remain densely granular. Mitotic division is still an active process, and migration is dorsal as previously mentioned. The general outline of the adrenal is becoming well established at this time.

At the end of the sixth day of incubation the cells have become more compact than they were in the preceding stage. They are grouped in masses on each side of the dorsal aorta and are becoming less constant in shape. Heretofore there was an active migration connected with an active mitotic division, now migration has practically ceased but division is still active, hence those cells in the central part of the

gland are subjected to pressure and are irregular in shape, while those on the periphery are more regular. At this stage the cortical cells have been pushed to the lateral margin of the mesonephros. The cells of the mesonephros resemble the cortical cells at this stage, often to such an extent that it is almost impossible to differentiate them. Such a condition no doubt is responsible for the earlier beliefs that the cortex was derived from the epithelium of the mesonephros. Had the investigations been confined to younger embryos this erroneous belief would have been avoided. Hays says that minute examination shows a thin layer of flattened mesenchyme cells between the two bodies. In my work I could not distinguish any boundary, both groups of cells seeming to lie in juxtaposition with no separating elements. The cytoplasm of the cortical cells is still densely granular. (Fig. 3.)

During the seventh day of incubation the size of the gland has noticeably increased. This is due in part to the invasion of sympathetic cells which have entered and are undergoing division; in part to the continued division of the cortical cells and in part to the formation of sinusoids within the gland. The sinusoids are formed from inpushings of the subcardinal vein. The cortical cells are arranged in irregular masses containing from 8 to 26 cells in each. These masses or cords of cells are less compact than they

were in the preceding stage. No apparent reason could be found to explain the fact of their loosening up. There may be a correlation between this fact and the appearance of the sinusoids at this time. Further work is necessary on this phase of the problem, and it is the intention of the writer to carry on further investigations in regard to this phenomenon and in regard to the character of the granules which are found in the cells.

During the eighth day of incubation the cords of cortical cells loosen still more; the cells divide rapidly, and the cords assume very irregular shapes. The general outline and embryonic size of the gland is well established at this time.

During the ninth day there is little increase noted in the size of the gland. More blood spaces have appeared and the cell masses have become more dense and compact again. The medullary cells are arranging themselves around the borders of the blood spaces, where as previously, they have been scattered cords of cells without regard to the sinusoids. It is barely possible that a hormone is secreted by the blood at his time which stimulates them to migrate. Further work is necessary to establish this theory. From this period until the end of incubation there is little variation in the general form of the gland. The most noticeable change is a

continued shifting and arranging of the medullary cells around the periphery of the sinusoids.

Origin and growth of the Medullary Substance.

About the fifth day of incubation, large cells which have detached themselves from the ventral trunk of the sympathetic nerves, migrate ventrally on each side of the dorsal aorta. (Fig. 4.) In most cases the cells migrate singly but in a few cases there are clusters of four and five. While these tend to assume a position mesial and ventral to the dorsal aorta there are some which turn off to the right or left as the case may be and enter the loose cords of cortical cells. As development goes on, more cells enter. Some may be seen adhering to the surface or periphery of the gland. At this stage of development the nucleus of the cells, which are the anlagen of the medullary substance, are round and clear, and the cytoplasm is finely granular. No change is apparent between the cells which have migrated among the cords of cortical cells and those which have continued farther ventrally to form the prevertebral sympathetic plexuses.

During the next day of incubation no changes are observable with the exception that more and more sympathetic cells are to be seen in the mesenchyme surrounding the gland. At this stage sympathetic cells are to be found on all sides of

the gland, singly and in scattered groups.

During the seventh day of incubation those cells which had migrated into the cortical substance are changing in shape, and a differentiation in the nuclei is taking place. The nuclei are becoming granular. These granules consist for the most part of small spherical bodies although a few rod shaped inclusions are present. The cells are losing their characteristic rotundity and are assuming irregular shapes. This is due to the fact that the cells are dividing but not migrating, hence the cytoplasm is pushed or crowded into all available corners. The daughter cells are smaller than the parent cells and there is a tendency for the arrangement to be in groups or cords within the cortical substance. The medullary cells at this stage are easily distinguished from the cortical cells because they have a deep staining property and appear dark to black in the microphotographs. (Fig. 5.)

During the eighth day of incubation the medullary cells have increased in number and are arranged in well defined cords within the cortical substance. (Fig. 6.) The mesenchyme surrounding the gland contains fewer sympathetic cells. There is a tendency for the medullary cords to be less compact. This tendency is possibly a presymptom of the change which ensues.

This change which occurs during the ninth and tenth days of incubation affects the internal arrangement of the medullary

cords. They now break down but do not return to their former condition of isolated cells, instead they arrange themselves in cords around the periphery of the blood spaces in the gland. Mention of this fact was previously made under the discussion concerning the development of the cortical cells and the first appearance of blood spaces, but no explanation was offered. It is possible that a hormone is secreted at this time which activates the medullary substance to migration and subsequent arrangement. If this is so, then the loosening of the cords in the previous stage is explained. Further work is necessary on this phase of the problem. From observations it appears that at this stage an activating agent of some kind is present, but of what kind is a matter of conjecture. Further observations lead to the discovery that only those groups of medullary cells which are in direct contact with the blood spaces act in this manner. The groups of cells which are not in contact with the blood spaces do not migrate to them. Because of this fact the cords of medullary cells are irregular in position, that is, they are scattered among the cortical cells promiscuously in groups and cords of varying numbers of cells.

From this time to the end of incubation no further changes are apparent, in size or form of the gland. It has reached its maximum embryonic size at this period.

During the 17th and 18th days the gland has the appear-

anse of numerous islets of cells among blood spaces. (Fig. 7.) If any regularity is to be observed at this stage of the development it is in the manner in which the cells are grouped around the blood spaces.

The connective tissue has grown slowly up to this period, (the 18th day of incubation). Now it is forming a sheath around the gland and can be seen entering among the cords of cells. Within the gland it breaks up quickly and but a few scattering strands may be seen. A dense layer of tissue is forming around the periphery of the gland giving evidence of a capsule to be formed at a later period. (Fig. 8.)

In the adult gland no change is seen from that at the end of incubation, with the exception of increase in size. In this case the increase is due to the cortical substance which is greater in proportion to the medullary substance than in the case of the embryo.

CONCLUSIONS

From the above observations my conclusions are: that the anlagen of the cortical cells of the adrenal gland are budded off the peritoneal epithelium; later sever connections with the mother cells and proliferate by active mitosis until about the end of the ninth day of incubation, when the gland has reached its embryonic growth. The anlagen of the cortical cells which are round at the beginning of development later lose their rotundity and assume irregular shapes without regard to polarity. Loose cords of cells are formed about the fifth day of incubation, at which time the invasion of the anlagen of the medullary cells takes place. Later these cords loosen up about the time of formation of blood spaces within the gland, and become more dense and compact again when the blood spaces have reached their maximum development.

The analgen of the medullary substance is formed by cells which detach themselves from the ventral trunk of the sympathetic nerves and migrate into the loose cords of cortical cells about the fifth day of incubation. No change is apparent between the cells which are the anlagen of the medullary substance and the cells which go to form the prevertebral sympathetic plexuses until about the seventh day of incubation, at which time a change appears in the size of the

cell and granulations of the nuclei. During the eighth, ninth, and tenth days changes appear in arrangement of the cords or groups of medullary cells around the blood spaces. From this time on no further changes are apparent in size or form of the gland.

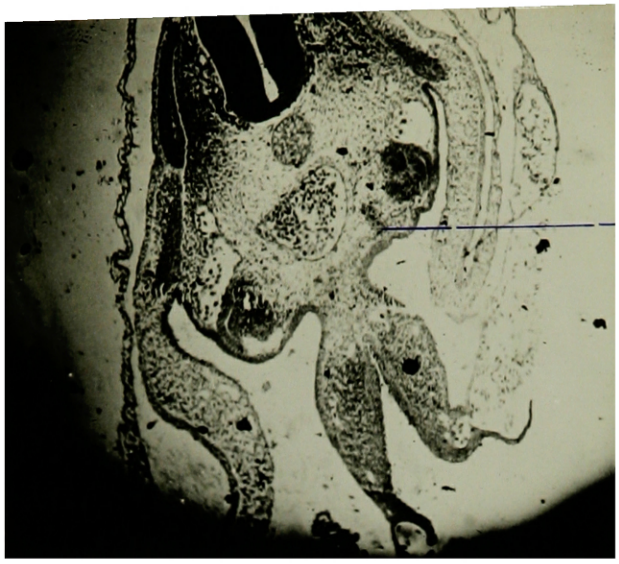

Fig. 1. Am. Bud of cells which give rise to cortical substance

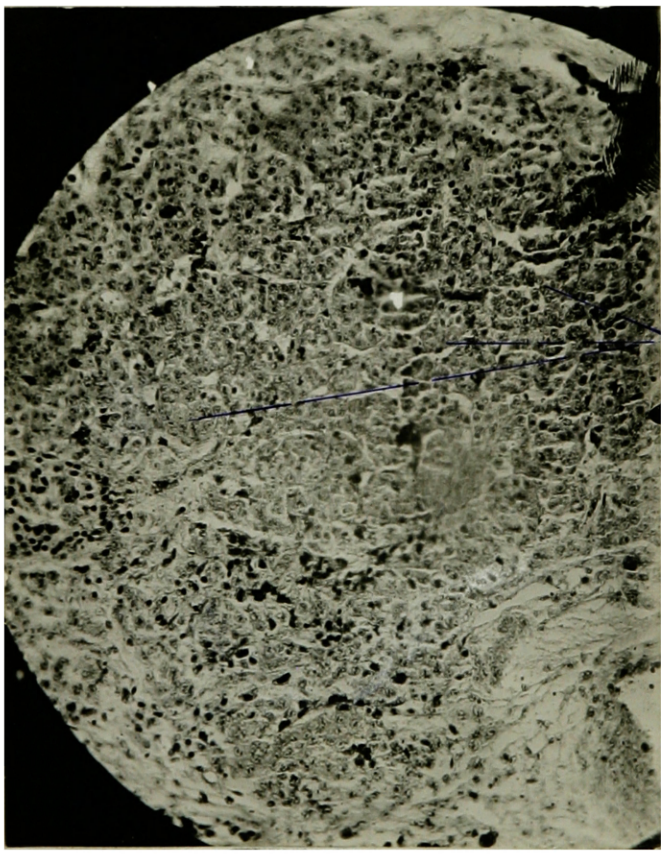

Fig. 2. C. C. Cords of cortical cells.

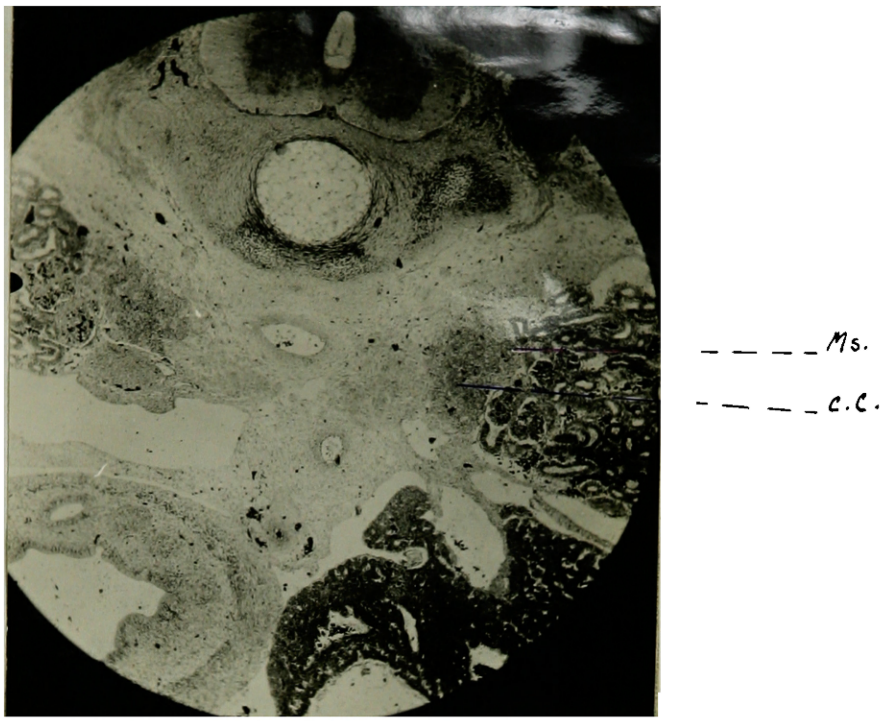

Fig. 3. C. C. Cortical Cells. Ms. Mesonephros.

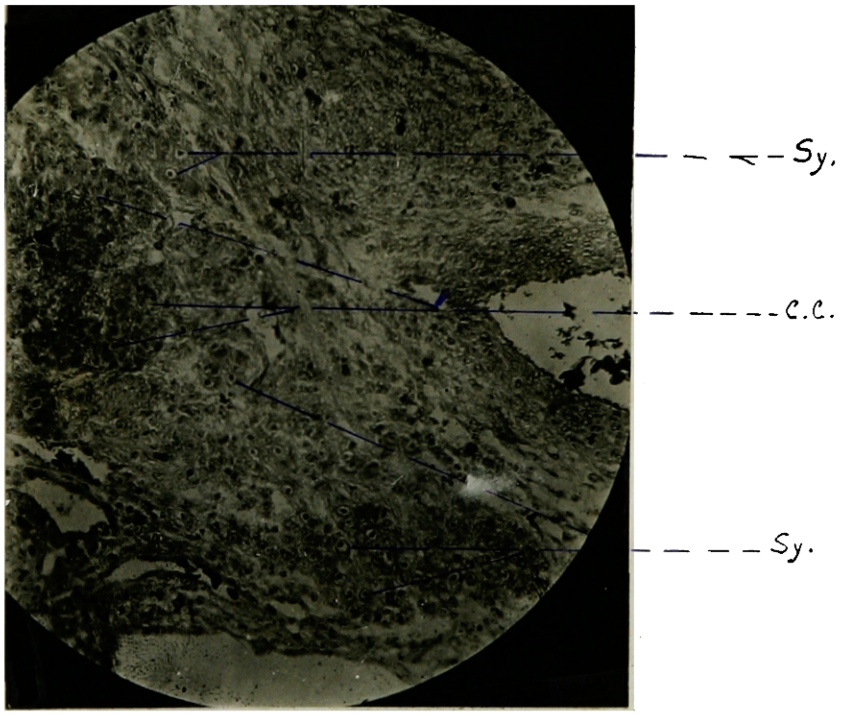

Fig. 4. C. C. Cortical cells. Sy. Sympathetic Nervecells.

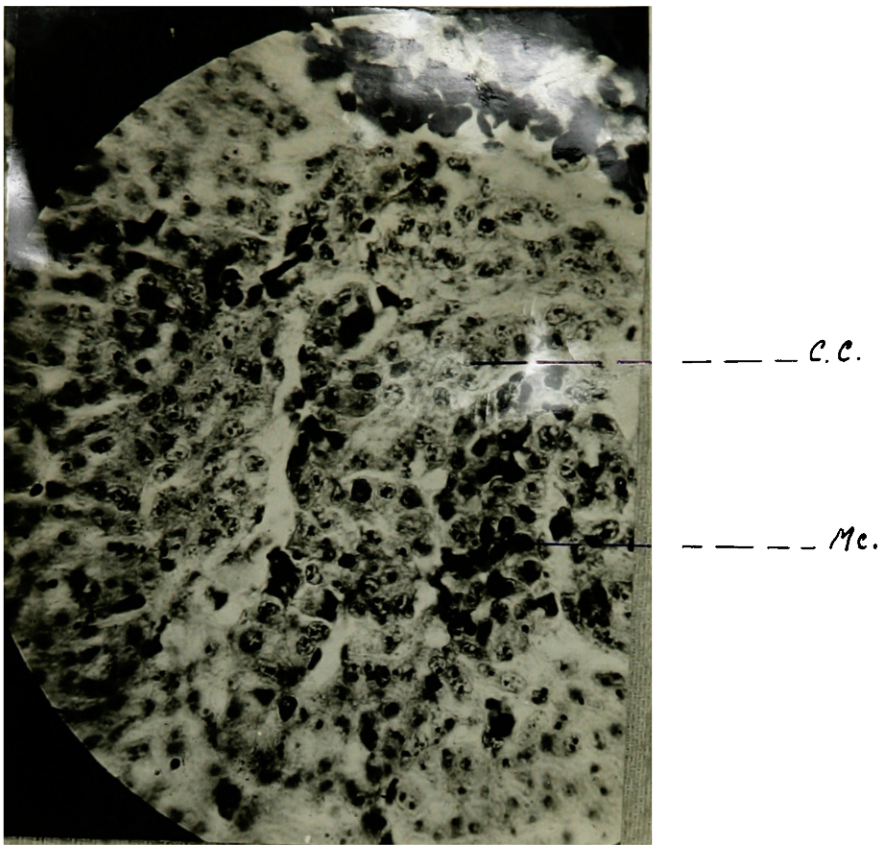

Fig. 5. C. C. Cortical cells. Mc. Medullary cells.

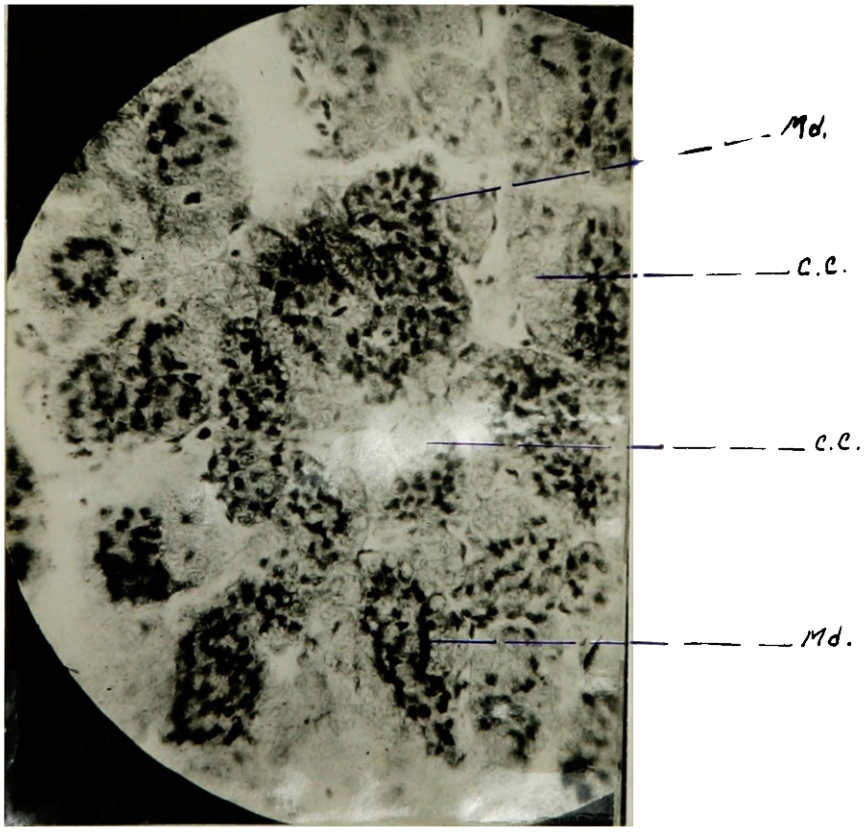

Fig. 6. C. C. Cortical Cells. Md. Cords of Medullary Cells.

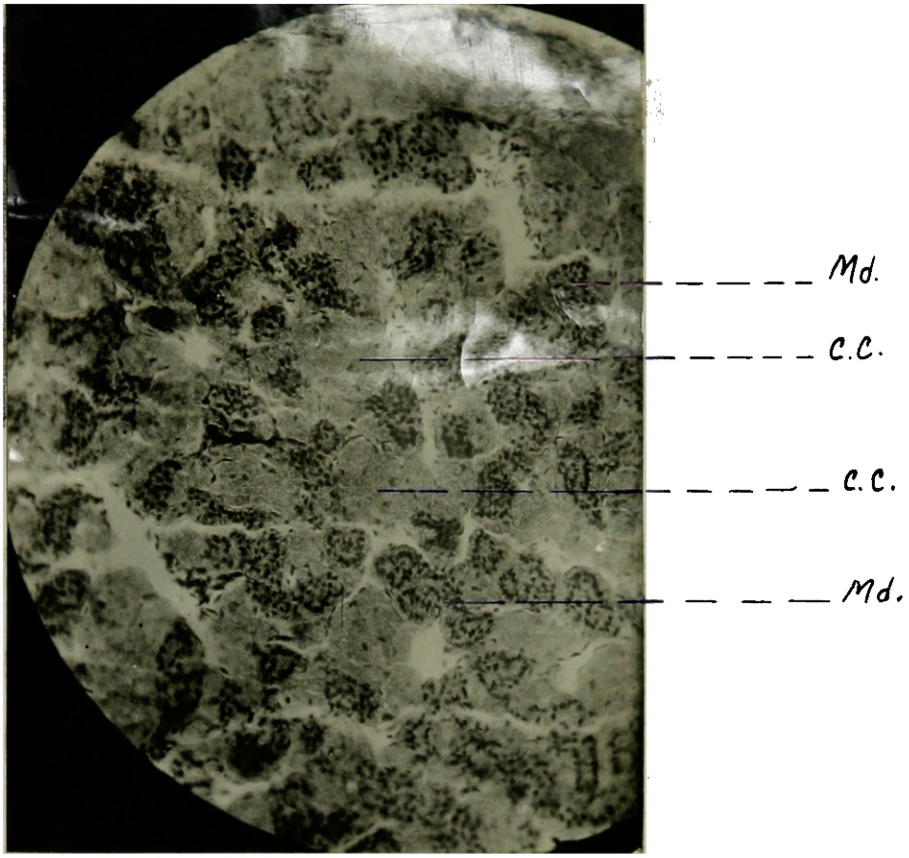

Fig. 7. C. C. Cortical Cells. Md Groups of Medullary Cells.

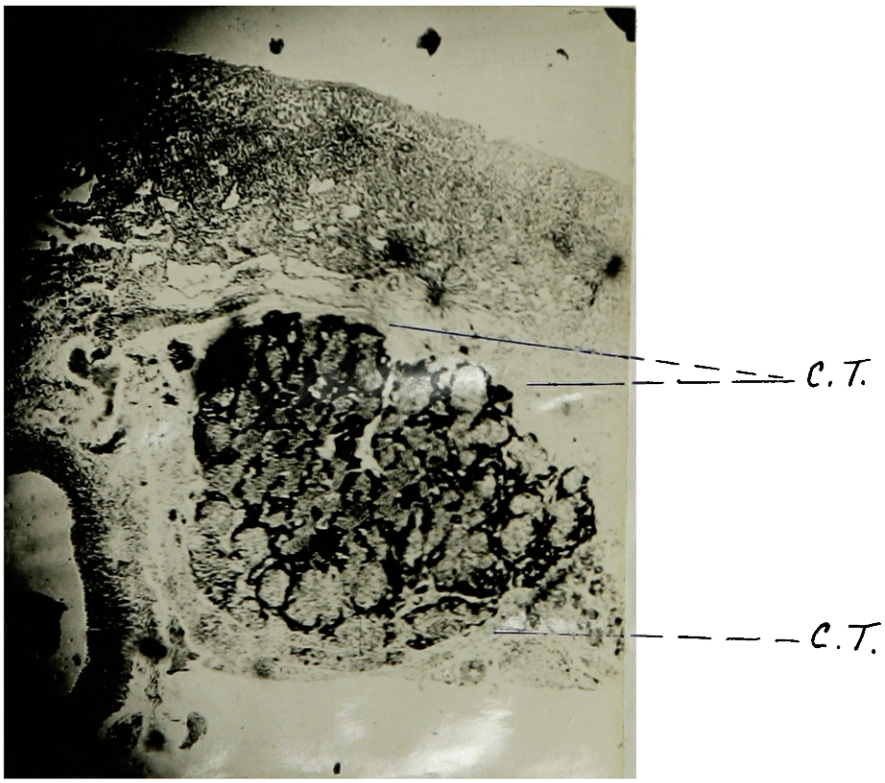

Fig. 8. C. T. Connective Tissue forming a capsule.